

Supplementary Material

***Vitis* flower sex specification acts downstream and independently of the ABCDE model genes**

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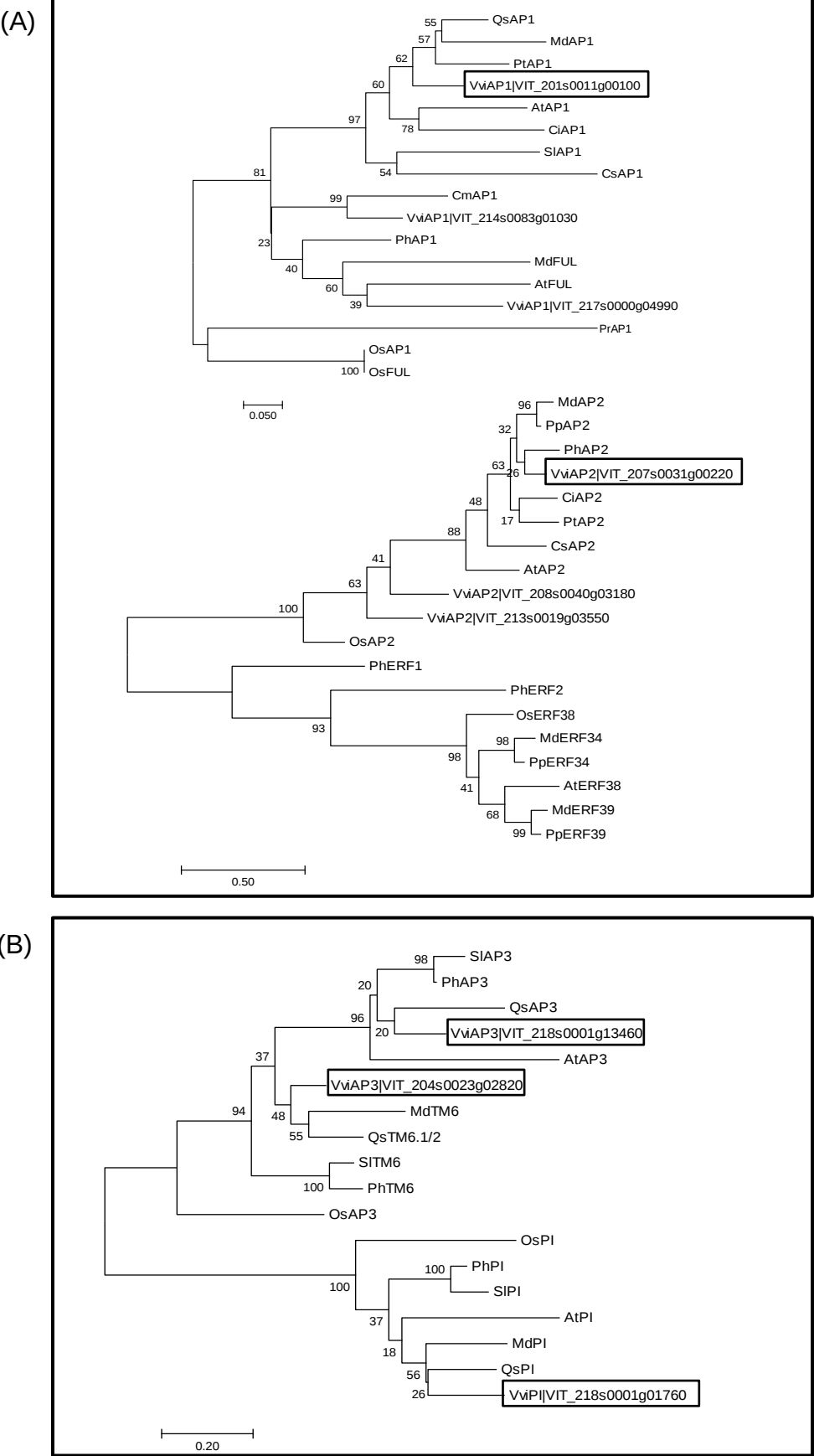
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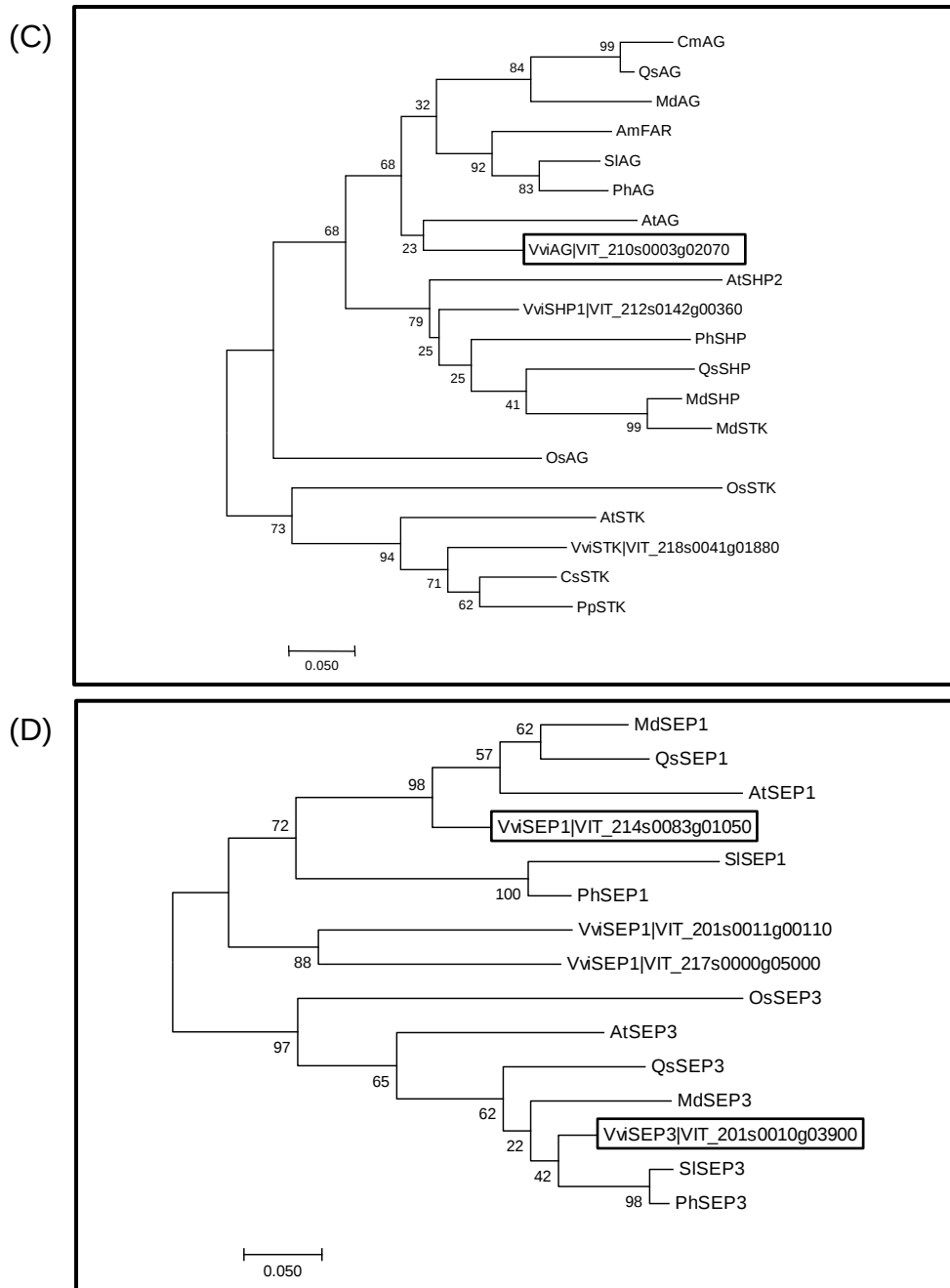
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Supplementary Figures and Tables:





Supplementary Figure 1. Phylogenetic analysis of flower homeotic genes from different plant species. *Vitis vinifera vinifera* (Vvi) ABCDE amino acid sequences were deduced using the cDNA sequence of gene ID from CRIBI (<http://genomes.cribi.unipd.it/grape/>) and translated using the ExPASy translate tool (<http://web.expasy.org/translate/>). Phylogenetic analysis of homeotic genes protein sequences was performed with the Maximum Likelihood method through MEGA (Molecular Evolutionary Genetics Analysis) version 6. The bootstrap consensus tree was inferred from 1000 replicates and represents the evolutionary history of the protein analyzed. (A) APETALA1 (AP1) and APETALA2 (AP2); (B) APETALA3 (AP3), TOMATO MADSBX6 (TM6) and PISTILLATA (PI); (C) AGAMOUS (AG), SHATTERPROOF (SHP) and SEEDSTICK (SKT); (D) SEPALLATA 1 and 3.

Am - *Antirrhinum majus*; At - *Arabidopsis thaliana*; Ci - *Citrus sinensis*; Cm - *Castanea mollissima*; Cs - *Cucumis sativus*; Md - *Malus domestica*; Os - *Oryza sativa*; Ph - *Petunia hybrida*; Pt - *Populus trichocarpa*; Pp - *Prunus persica*; Pr - *Pinus radiata* Qs - *Quercus suber*; Sl - *Solanum lycopersicum*; Vvi - *Vitis vinifera vinifera*. Accession numbers for amino acids sequences are presented in Supplementary Table 2 and 3.

VviAP3|VIT_204s0023g02820
MCCLKSKDPTPHVITLIPKEPFHGFQHSQSLHYNLTLATYPIQKLIFLFPSSSSSSSTYCALEPHYLILEK
KEGSKARKMGRGKIEIKRIENPTNRQVTYSKRRNGIFKKAQELTVLCDAKVSLIMFSNTGKFHEYTSPTIT
TKKVYDQYQKTLGIDLWSSHYERMQENLRKLKEINNKLRRERQRMGEDLGDSIEDLRGLEQKMDASL
GLVRERKYHVIKTQTETYSKVRNLEEQHGNLLLNFEAKCDDPHYGLVENDGDYESAVAFANGASNL
AFRLHOAHPNLHHDGGYGS~~DLRLA~~

Supplementary Figure 2. Protein sequence and alignment of VIT_204s0023g02820 and VIT_218s0001g13460. Protein sequence and alignment of the *VviAP3* genes according to Expasy translation tool (<http://web.expasy.org/translate/>) using the cDNA sequence from CRIBI (<http://genomes.cribi.unipd.it/grape/>) for both genes annotated as *VviAP3*. It is possible to observe that the *VviAP3* with the ID VIT_218s0001g13460 display the C-terminal sequence DLTFTLLE while the *VviAP3* with the ID VIT_204s0023g02820 display the C-terminal sequence DLRLA corresponding to the euAP3 and paleoAP3 lineage, respectively.



Supplementary Figure 3. Protein sequence alignment of *SHATTERPROOF* genes from *Vitis* (*Vvi*) and *Arabidopsis* (*At*). (A) The alignment between *VviSHP1* (VIT_212s0142g00360) and *AtSHP1* revealed an amino acid identity of 70%. (B) The alignment between *VviSHP1* and *AtSHP2* revealed an amino acid identity of 82%. The *VviSHP1* is closer to *AtSHP2* than to *AtSHP1*.

Supplementary Table 1. Time of probe hydrolysis at 60° C according to the fragment size. F, forward primer; R, reverse primer. The template length includes the sequence from the T7 or SP6 binding site to the polylinker.

Gene ID	Probe	Primer sequence (5'-3')	Template length (bp)	Time of hydrolysis (min)
VIT_201s0011g00100	<i>VviAP1</i>	F- AAAGATGGGAAGAGGTAGGGTT R- AGTGCCTTTGGCTTCTTTGTA	448	18
VIT_207s0031g00220	<i>VviAP2</i>	F- GTCGGAAATGGGGACTACTT R- ATTGTTCTGCTTGGATGCGG	724	20
VIT_218s0001g13460	<i>VviAP3</i>	F- CTTTGTGATGCTAAGGTTTC R- ACGATGGTGGGATTCAATTG	817	12
VIT_218s0001g01760	<i>VviPI</i>	F- AAGCAACAGGCAGGTGAC R- ATAAGTTTTGAGGGTAATGG	823	80
VIT_204s0023g02820	<i>VviTM6</i>	F- CACCGTTCTTTGTGATGCTAA R- GAACCAGACAATAGAG	905	84
VIT_210s0003g02070	<i>VviAG</i>	F- TCCGTTTCTGAAGCCAA R- ATCTGATTCTACTTATGCCT	278	-
VIT_205s0049g00070	<i>VviSUP</i>	F- GCAACAACCACAAGGTCAAG R- GGAACCATCCACACCCCATT	514	5
VIT_214s0083g01050	<i>VviSEP1</i>	F- GGGAAAGTGGTGAGCAGAGTA R- CCATTGACATTTTGGGCATTA	381	-
VIT_201s0010g03900	<i>VviSEP3</i>	F- TATCCACAAGGGAGGCCCTG R- GCAGGCCCCAGTGTGAATAAC	571	14

Supplementary Table 2. Accession numbers for *Vitis* gene names. Abbreviations are the following: *AP1*, *APETALA1*; *AP2*, *APETALA2*; *AP3*, *APETALA3*; *PI*, *PISTILLATA*; *AG*, *AGAMOUS*; *FUL*, *FRUITFULL*; *SHP*, *SHATTERPROOF*; *SKT*, *SEEDSTICK*; *SEP*, *SEPALLATA*; *TM6*, *TOMATO MADSBOX6*.

<i>Vitis</i> species	Gene (ID)
<i>VviAPETALA1</i>	VIT_217s0000g04990
	VIT_214s0083g01030
	VIT_201s0011g00100
<i>VviAPETALA2</i>	VIT_207s0031g00220
	VIT_213s0019g03550
	VIT_208s0040g03180
<i>VviAPETALA3</i>	VIT_218s0001g13460
<i>VviPISTILLATA</i>	VIT_218s0001g01760
<i>VviTM6</i>	VIT_204s0023g02820
<i>VviAGAMOUS</i>	VIT_210s0003g02070
<i>VviSHATTERPROOF</i>	VIT_212s0142g00360
<i>VviSEEDSTICK</i>	VIT_218s0041g01880
<i>VviSEPALLATA1</i>	VIT_201s0011g00110
	VIT_214s0083g01050
	VIT_217s0000g05000
<i>VviSEPALLATA3</i>	VIT_201s0010g03900,
	VIT_214s0068g01800

Supplementary Table 3. Accession numbers for non *Vitis* species amino acids sequences used for phylogenetic analysis. Abbreviations of gene names: *AP1*, *APETALA1*; *AP2*, *APETALA2*; *AP3*, *APETALA3*; *PI*, *PISTILLATA*; *AG*, *AGAMOUS*; *FUL*, *FRUITFULL*; *SHP*, *SHATTERPROOF*; *SKT*, *SEEDSTICK*; *SEP*, *SEPALLATA*; *TM6*, *TOMATO MADSDOX6*.

Species	Gene	Acession number (protein)
<i>Arabidopsis</i>	<i>AtAP1</i>	NP_177074.1
	<i>AtFUL</i>	Q38876.1
	<i>AtAP2</i>	P47927.1
	<i>AtERF38</i>	NP_181113.1
	<i>AtAP3</i>	NP_191002.1
	<i>AtPI</i>	NP_197524.1
	<i>AtAG</i>	NP_567569.3
	<i>AtSHP2</i>	AAU82079.1
	<i>AtSTK</i>	NP_001078364.1
	<i>AtSEP1</i>	NP_001119230.1
	<i>AtSEP3</i>	NP_564214.2
<i>Cucumis sativus</i>	<i>CsAP1</i>	XP_011650331.1
	<i>CsAP2</i>	XP_004149686.1
	<i>CsSTK</i>	NP_001267506.1
<i>Malus domestica</i>	<i>MdAP1</i>	ABG85297.1
	<i>MdFUL</i>	XP_008374663.1
	<i>MdAP2</i>	NP_001280879.1
	<i>MdERF34</i>	XP_008347085.1
	<i>MdERF39</i>	XP_008369428.1
	<i>MdTM6</i>	XP_008346633.1
	<i>MdPI</i>	NP_001280926.1
	<i>MdAG</i>	NP_001315863.1
	<i>MdSHP</i>	NP_001280918.1
	<i>MdSTK</i>	NP_001280758.1
	<i>MdSEP1</i>	AAC25922.1
	<i>MdSEP3</i>	NP_001280756.1
<i>Oryza sativa</i>	<i>OsAP1</i>	XP_015631033
	<i>OsFUL</i>	XP_015631034.1
	<i>OsAP2</i>	Q10BG6
	<i>OsERF38</i>	XP_015634439.1
	<i>OsAP3</i>	XP_015641661.1
	<i>OsPI</i>	XP_015640709.1
	<i>OsAG</i>	XP_015632490.1
	<i>OsSTK</i>	XP_015621182.1
	<i>OsSEP3</i>	XP_015648762.1

Supplementary Table 3 (cont). Accession numbers for non *Vitis* species amino acids sequences. Abbreviations of gene names: AP1, APETALA1; AP2, APETALA2; AP3, APETALA3; PI, PISTILLATA; AG, AGAMOUS; FUL, FRUITFULL; SHP, SHATTERPROOF; SKT, SEEDSTICK; SEP, SEPALLATA; TM6, TOMATO MADSBX6.

Species	Gene	Acession number (protein)
<i>Petunia hybrida</i>	<i>PhAP1</i>	AAF19721.1
	<i>PhAP2</i>	Q9XHD4
	<i>PhERF2</i>	ADP37417.1
	<i>PhERF1</i>	ADP37416.1
	<i>PhTM6</i>	AAF73933.1
	<i>PhAP3</i>	Q07472.1
	<i>PhPI</i>	CAA50549.1
	<i>PhAG</i>	Q40885.1
	<i>PhSHP</i>	CAA48635.1
	<i>PhSEP1</i>	AAQ72498.1
	<i>PhSEP3</i>	Q03489.2
<i>Prunus persica</i>	<i>PpAP2</i>	AEB92231.1
	<i>PpERF39</i>	XP_007209502.1
	<i>PpERF34</i>	XP_007218799.2
	<i>PpSTK</i>	ABQ85556.1
<i>Populus trichocarpa</i>	<i>PtAP1</i>	AAT39554.1
	<i>PtAP2</i>	POPTR_0007s10780.1
<i>Solanum lycopersicum</i>	<i>SIAP1</i>	NP_001234665.1
	<i>SITM6</i>	NP_001311309.1
	<i>SIAP3</i>	NP_001234077.2
	<i>SIP1</i>	ABG73411.1
	<i>SIAG</i>	NP_001266181.1
	<i>SISEP1</i>	AAM33104.2
	<i>SISEP3</i>	NP_001234384.1
<i>Citrus sinensis</i>	<i>CiAP1</i>	NP_001275828.1
	<i>CiAP2</i>	XP_006494466.1
<i>Quercus suber</i>	<i>QsAP1</i>	XP_023910685.1
	<i>QsTM6.1/2</i>	XP_023911257.1
	<i>QsAP3</i>	XP_023924416.1
	<i>QsPI</i>	XP_023887186.1
	<i>QsAG</i>	XP_023894634.1
	<i>QsSHP</i>	XP_023917960.1
	<i>QsSEP1</i>	XP_023912244.1
	<i>QsSEP3</i>	XP_023899435.1
<i>Castanea mollissima</i>	<i>CmAP1</i>	AAZ77749.1
	<i>CmAG</i>	AAZ77747.1
<i>Pinus radiata</i>	<i>PrAP1</i>	AAD09207.1
<i>Antirrhinum majus</i>	<i>AmFAR</i>	CAB42988.1